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ALLELOPATHIC EFFECT OF *TITHONIA DIVERSIFOLIA* AND *CHROMOLAENA ODORATA* ON THE GERMINATION, GROWTH AND CHLOROPHYLL ACCUMULATION OF *HIBISCUS SABDARIFFA* (L.)

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ABSTRACT

Laboratory and soil cultured experiments were conducted to evaluate: the allelopathic activities of the fresh shoot aqueous extracts of *Tithonia diversifolia* (FSET) and *Chromolaena odorata* (FSEC) at concentrations 50%, 80% and 100% on the germination, radicle and plumule growth of *Hibiscus sabdariffa* plants, investigate the effects of 100%FSET and 100%FSEC on the growth, chlorophyll pigments, ascorbic acid and protein contents of this plant. Seed germination and juvenile seedling growth were reduced with the lowest concentration of the different extracts. The radicle growth was more inhibited than the plumule growth. The plant extracts had a concentration-dependent reduction of the seedling growth of the target crop. The FSET was more phytotoxic than FSEC. The soil-cultured experiment showed that both FSET and FSEC significantly enhanced the number of leaves, chlorophyll b, total chlorophyll, ascorbic acid and protein contents of older *H. sabdariffa* plants. In addition to these, FSEC significantly promoted the shoot height, stem girth, leaf area, leaf area ratio and shoot fresh and dry weights of these plants. This study has shown that FSET and FSEC could play differing allelopathic physiological role, depending on the medium of growth and age of the target plant. It was noted that the phytotoxicity of *C. odorata* and *T. diversifolia* got degraded in the soil such that the extracts were stimulatory to the growth of the target plants. This finding suggested that *C. odorata* could serve as green manure or bio-fertilizer to boost the growth and productivity of established *H. sabdariffa* plant on the field.

KEYWORDS: Allelopathic, Ascorbic Acid, *Chromolaena odorata*, Concentration-Dependent, *Hibiscus sabdariffa*, *Tithonia diversifolia*

INTRODUCTION

Allelopathy refers to any process involving secondary metabolites produced by plants, micro-organisms, fungi etc. that positively or negatively influence the growth and development of agricultural and biological systems (International Allelopathy Society, 1996). A key concept in allelopathy is that these secondary metabolites or allelochemicals are transferred through the environment from one organism to the other (Einhellig, 1995). These allelochemicals are released into the environment (atmosphere or rhizosphere) in ample quantities by means of volatilization, leaching, decomposition of residues, root exudation etc. and if persistent long enough could either stimulate or inhibit the growth and physiological processes of the neighbouring or successional plant (Putnam, 1988; Chou, 1998 and Inderjit and Keating 1999). Nasrine *et al.*, (2011) stated that allelopathy includes plant-plant, plant-microorganisms, plant-virus, plant-insects, and plant-soil-plant chemical interaction.

Several workers have reported on the allelopathic potential of common weeds on germination, seedling growth and yield of several crop species (Inderjit and Dakshini, 1998; Singh *et al.,* 2003; Kong *et al.,* 2007; Ilori *et al.,* 2010; Otusanya, 2014). Chivinge, (1985) and Hagin, (1989) found that soil extracts or weed residues and decompose of organism possess allelopathic potential to increase the growth and yield of some crops. Similarly, allelopathic water extract applications at lower concentrations have been observed to stimulate germination and growth of different crops (Anwar *et al.,* 2003; Cheema *et al.,* 2012). Chon *et al.,* (2003a, b) reported that aqueous extracts of some plants from Asteraceae family at low concentration increased the root length of alfalfa up to 13-33%. El-Rokiek *et al.,* (2006) showed that application of the rice straw aqueous extract at lower and moderate concentration significantly stimulated the shoot elongation and shoot biomass of soybean plants at both vegetative and flowering stages of growth. Rice hull extract had stimulatory effect on stem length of *Silybum marianum* and also enhanced the dry weight of *Echinochloa crusgalli* (Seyyednijad *et al.,* 2010). Abdalla, (2013) reported that fertilization of rocket plants (*Eruca vesicaria*) with 2% leaf and 3% twig aqueous extracts of *Moringa oleifera* potentially increased the plant height, fresh and dry herb weight, photosynthetic rates, stomatal conductance, chlorophyll a and b, carotenoids, total sugars, total protein, phenols, ascorbic acid, nitrogen, phosphorus, potassium, calcium, magnessium, iron as well as growth promoting hormones (auxins, gibberellins and cytokinins). According to Hawes *et al.,* (2003) and Dakora and Phillips, (2002), plants release secondary compounds into their environment that change soil chemistry, thus increasing nutrient uptake or protecting against metal toxicity. Niel and Rice (1971) reported that rhizosphere soil upon which *A. psilostachya* grows stimulated the growth of several plant species, including *Amaranthus retroflexus* that occurred in the same field. Soil application of *Nicotiana plumbagmifolia* leachate (25%) in maize field improved root and shoot length 4.15% and 18% respectively (Farooq *et al.,* 2013). The same authors stated that application of *Ipomoea cairica* aqueous leachate (0.025 g mL-1) to the root of *Brassica spp.* improved germination by 5.2% and root and shoot length by 3.7%

Tithonia diversifolia (Hemsl) A. Gray and *Chromolaena odorata* (L.) King and Robinson, both in the family Asteraceae are highly invasive environmental weeds due to their aggressive growth rate and heavy seed production (Muoghalu and Chuba, 2005; Zachariades *et al.,* 2009). These weeds are most prominent in the rain forest region of Nigeria, infesting severely the natural habitats and plantation crops. The release of the allelochemicals in these weeds into the environment during the rainy season has been reported to influence both physiological and biochemical characteristics of crops. For example, Akobundu (1987) observed that the growth of other plants was hampered in the areas where *C. odorata* grows. Tijani and Fawusi (1989) reported on the allelopathic activities of crude methanol extract of *C. odorata* on seed germination and seedling growth of tomato. Eze and Gill (1992) stated that *C. odorata* contains a large amount of allelochemicals especially in the leaves, which inhibit the growth of many plants in nurseries and in plantations. Rafiqul-Hoque *et al,* (2003) found that different concentrations of *C. odorata* leaf aqueous extract significantly inhibited the germination, root and shoot elongation and development of lateral roots of *Cicer arietinum, Brassica juncea, Cucumis sativus, Phaseolus mungo, Raphanus sativus* and *Vigna unguiculata*. These authors emphasized that the inhibitory effect was proportional to the concentration of the extracts, that is, higher concentration had the stronger inhibition whereas in some cases, the lower concentration showed stimulatory effect. Adetayo *et al.* (2005) observed 14% and 8% reduction in seed germination of cowpea and soybean treated with the water extract of *C. odorata* leaves and stems respectively. According to Otusanya *et al.* (2008), water root exudates of *T. diversifolia* significant inhibited the germination, growth and chlorophyll accumulation of tomato. On the other hand, Ilori *et al.* (2010) showed that the germination of cowpea seeds was significantly enhanced through treatment with the fresh shoot aqueous extract of *T. diversifolia* and *Helianthus*

annuus. Application of fresh shoot aqueous extract of *C. odorata* significantly enhanced the shoot height, leaf area, fresh and dry weight of *Celosia argentea* (Ilori *et al.*, 2011). The recent findings of Otusanya *et al.*, (2014) showed that the root exudates of *T. diversifolia* significantly inhibited the germination, growth and chlorophyll contents of *Amaranthus dubius.*

Hibiscus sabdariffa L. (Malvaceae) commonly known as Roselle is widely grown in tropics and subtropics of both hemispheres and has become naturalized in many areas of America and Africa (Morton, 1987). The thick, red and fleshy cup-shaped calyces of the flower are consumed worldwide as a cold beverage and as a hot drink (Sour tea) while the green and fleshy cup shaped calyces are used as soup condiment in southwest Nigeria. The extracts from this plant served as remedies for high blood pressure, liver diseases and fever in folk medicine (Ross, 2003; Wang *et al.*, 2000). The red anthocyanin pigments present in their calyces are also used as food colouring agents. Considering the numerous usefulness of this crop plant, the study was carried out to evaluate the effects of different concentrations (50%, 80% and 100%) of the aqueous extracts of *T. diversifolia* (FSET) and *C. odorata* (FSEC) on the germination, juvenile seedling growth of the crop plant and also to determine the influence of the undiluted extract (100%FSET and 100%FSEC) of both weeds on the growth, chlorophyll, ascorbic acid and protein accumulation in older (> two weeks) *H. sabdariffa* plants.

MATERIALS AND METHODS

Experimental Site and Materials Sources

The experiment was carried out at the Department of Botany, Obafemi Awolowo University (O.A.U.) Ile-Ife, Nigeria. The seeds of *H sabdariffa* and *T. diversifolia* were collected from National Horticultural Research Institute (NIHORT) Ibadan and along Ede road, near the O.A.U. main campus gate respectively. Young seedlings of *C. odorata* were collected from the site of the Botany Department Afforestation Scheme, O.A.U. Ile-Ife.

Preparation of the Fresh Shoot Aqueous Extract

Extraction procedures were carried out according to the method of Ahn and Chung (2000). 360 g of the fresh shoot of *T. diversifolia* (FSET) and *C. odorata* (FSEC) were harvested separately at vegetative stage and cut into small chips of about 4cm length. The chips were soaked in 5 Litre of distilled water for 12 hours and filtered through cheese cloth to remove the fibre debris and finally filtered through Whatman No. 1 filter paper to have a 100% concentration of the fresh shoot aqueous extract (100% FSE). The aqueous extracts of 50% and 80% concentrations were prepared by diluting the parent FSE with distilled water.

Seed Germination and Seedling Growth

H. sabdariffa seeds were randomly selected for uniformity on the basis of size and soaked for five minutes in 5% sodium hypochlorite to prevent fungal growth. Thereafter, the seeds were rinsed in running tap water for 5 mins and then thoroughly washed in double distilled water. Ten of these seeds were germinated in sterilized Petri-dishes lined with Whatman No 1 filter paper. The filter paper was moistened with 10 mL of the respective graded concentrations of fresh shoot aqueous extracts (100%, 80%, 50% FSET and 100%, 80%, 50% FSEC) in treatments and distilled water in control. Each treatment had six replicates. The Petri-dishes were incubated at room temperature (25 ± 2^0C) for 14 days. Emergence of 1mm of the radicle was used as the criterion for germination. Germination percentages were recorded and the radicle and plumule lengths were measured daily over 14 days of culture.

Soil Culture Experiment

Ten surface sterilized seeds were sown in experimental pots (55 cm diameter × 75 cm depth) that were filled with homogenous top humus soil. Each experimental pot had six holes perforated at the bottom for good drainage. All the pots were initially irrigated with 600 mL of water on a daily basis. At two weeks, the plants in each pot were thinned down to six uniform plants per pot. The pots were then allocated to the control and two different treatments, that is, fresh shoot aqueous extract of *T. diversifolia* (100%FSET) and fresh shoot aqueous extract of *C. odorata* (100%FSEC). The pots were arranged in a complete randomized design. Thereafter, the pots in the control regime were supplied with 600 mL of water daily while the pots in the treatment regimes were supplied with 600 mL of the appropriate aqueous extract daily. For zero day, plants were harvested just before the treatment started. Thereafter, harvesting of the plant was on weekly interval for a period of six weeks. Recording of the following growth parameters (shoot height, stem girth, number of leaves) data was carried out according to standard methods. The leaf area was determined using the method of Pearcy *et al.,* (1989) and the leaf area ratio calculated. Five shoots in each regime were weighed on Mettler Toledo balance to obtain the fresh weight. The shoots were then packaged separately in envelopes and dried to constant weight at 80^0C in a Gallenkhamp oven (Model IH-150) to obtain the dry weight. Chlorophyll contents of the fresh shoot were extracted with 80% acetone and quantified following the procedure of Comb *et al.,* (1985). Ascorbic acid and protein contents were determined according to the titrimetric method and micro-Kjeldahl nitrogen method respectively as described by AOAC (2000). Ascorbic acid in the sample was calculated using the formula below:

$$\text{Ascorbic acid in the Sample} = \frac{X \times Y \times \text{Titre of Sample}}{gram\ of\ Sample}$$

X is the ascorbic acid quantity equivalence of 1ml dichloro-indolephenol

Y is the ratio of the quantity (mL) of the extraction solution used for extracting the sample to the quantity (mL) taken for titration.

The percentage crude protein accumulation in the shoot of *H. sabdariffa* was estimated using the formulae below.

$$\%\ \text{Total Nitrogen} = \frac{(A-B) \times N \times 14.01 \times 100}{\text{gram of Sample} \times 10}$$

% Crude Protein = % Total Nitrogen × 6.25

A = sample reading, B = blank reading; N = Normality of acid used for titration, 100 = conversion to % and 6.25 is the correction factor (F)

Statistical Analysis

All experiments were conducted in five replicates and the data obtained were subjected to analysis of variance (ANOVA). Differences between individual means were determined by least significant difference (LSD) test at 0.05 level of probability. Data were analyzed using SPSS

RESULTS

Seed Germination and Seedling Growth

The aqueous extracts of *T. diversifolia* (FSET) and *C. odorata* (FSEC) showed significant inhibitory effects on seed germination and seedling growth of *H. sabdariffa* at $p \leq 0.05$ (Table 1). The control treatment produced the highest

germination percent (97.5%). Using the 100%FSET and 100%FSEC treatments, germination were reduced by 34% and 22% respectively. The retardation of germination observed for 50%FSET was not significantly different from that of 100%FSEC at $p \leq 0.05$.

The plants in the control regime had the longest plumule (3.03 cm) and radicle (2.10 cm) lengths (Table 1). Higher concentrations of aqueous extract (80%FSET, 100%FSET and 100%FSEC) significantly retarded the growth of plumule and radicle at $p \leq 0.05$. The length of plumule and radicle of the 100%FSET seedlings was reduced to 0.57 and 0.34cm respectively while that of 100%FSEC dropped to 1.92 and 1.46cm respectively. Similarly, 0.78 and 0.50cm were recorded respectively for the plumule and radicle length of 80%FSET-treated seedlings while relatively higher values, 2.11 and 1.78cm were obtained for 80%FSEC-treated seedlings, therefore, the inhibitory effect of FSET was stronger than that of FSEC and this difference was statistically significant at $p \leq 0.05$. The retardation of germination and seedling growth followed the trend 100% FSET > 80%FSET > 50%FSET $\geq$ 100%FSEC > 80% FSEC > 50% FSEC.

Growth Parameters, Photosynthetic Pigment and Biochemical Constituents

Tables 2-4(a) show the effect of the aqueous extract of *T. diversifolia* (FSET) and *C.odorata* (FSEC) on the shoot height, stem girth, number of leaves, shoot fresh and dry weights, leaf area and leaf area ratio of *H. sabdariffa* plants. The 100%FSEC stimulated all the growth parameters while 100% FSET stimulated only the number of leaves on the test crop showing that FSEC possessed more stimulatory functions than the FSET on the field.

Chlorophyll a, chlorophyll b and total chlorophyll contents of the aqueous extract-treated plants were progressively increased throughout the experimental period (Tables 4b&c and 5a). The FSEC treatment significantly increased chlorophyll b and total chlorophyll accumulation by 298.41 and 97.41% respectively while the FSET application induced a significant increase of 303.93 and 91.03% on chlorophyll b and total chlorophyll respectively at $p \leq 0.05$.

The ascorbic acid and percentage protein contents in both the treated and the control *H. sabdariffa* plants is as shown in Table 5 (b&c). The accumulation of ascorbic acid was significantly increased by the application of both FSET and FSEC at $p \leq 0.05$. The highest stimulation of 87.71% was recorded for the 100%FSEC-treated plants, while application of 100%FSET accounted for 66.11% increase compared with the control. Similarly, application of FSET and FSEC stimulated significantly the protein contents of the test crop at $p \leq 0.05$ respectively. Both FSET and FSEC enhanced the accumulation of protein in the shoot of these plants by 58.47 and 66.94% respectively.

DISCUSSIONS

In this study, the different concentrations of FSET and FSEC were found to significantly reduce the germination of the seeds of *H. sabdariffa.* This is consistent with the findings of Ilori *et al.*, (2010) who reported that FSEC significantly reduced germination, plumule and radicle length of *Vigna unguiculata*. Similarly, Tefera (2002) reported earlier that 10% leaf aqueous extract of *Parthenium hysterophorus*, also in the family Asteraceae completely suppressed the germination of the seeds of *Eragostis tef.* Marharjan *et al.*, (2007) asserted that such inhibition was due to either prevention of the growth of the embryo or reduction of the rate of the mitotic process of the seeds by the allelochemicals in the aqueous extracts. Some other workers suggested that allelopathic inhibition of germination may be the result of inhibition of water uptake and alteration in the synthesis or activity of gibberellic acid by phenolic compounds present in the aqueous extracts (Einhellig, 1996; Olofsdotter, 2001; Tawaha and Turk, 2003; Otusanya and Ilori, 2012).

The inhibition of the juvenile seedling growth of *H. sabdariffa* was dependent on the concentration of the applied extracts. This agreed with Sahid and Sugau (1993) who had earlier emphasized that allelopathic agents were highly selectively absorbed and their effect was concentration dependent. Om *et al.,* (2002) reported that the higher concentration of weed extract (1:4) had more inhibitory effect than the lower concentration (1:8). Similarly, Turk *et al.,* (2003) observed that the inhibition of the radicle and plumule growth of wild oat increased with increasing concentration of the extract solution of the fresh plant parts of Black mustard. In this work, the growth of the radicle was more significantly inhibited than the plumule. This corroborates the findings of Caamal-Maldonado *et al.* (2001) who stated that 1% aqueous leachate of *Mucuna deeringiana*, *Canavalia ensiformis*, L*eucaena leucocephala* and *Lysiloma latisiliquum* exhibited strong phytotoxic effect on the radicle growth of barnyard grass, alegría and amaranth. The strong inhibition of the radicle by the aqueous extracts may be the consequence of direct contact of the radicle with allelochemicals in the extract which according to Sasinath Jha *et al.,* (2006) could interfere directly with respiration or oxidative phosphorylation.

In this study, though, the weeds extract applied retarded the germination and juvenile growth of *H. sabdariffa*, they however, enhanced the growth of older (> two weeks) soil cultured plants. This finding suggested that the response of the recipient plant to allelochemical stress could be dependent on the age of the target plant. In this case, the growth of the older soil cultured plants was not susceptible to the phytotoxicity of the extracts rather it was enhanced. The result was contrary to Rafiqul-Hoque *et al,* (2003) who found that different concentrations of *C. odorata* leaf aqueous extract significantly inhibited the root and shoot elongation and development of lateral roots of *Cicer arietinum, Brassica juncea, Cucumis sativus, Phaseolus mungo*, *Raphanus sativus* and *Vigna unguiculata*. Therefore, the response of crops to the allelopathic potential of *C. odorata* could be species dependent. In fact, Bhadoria (2011) considered the process of allelopathy as being genetically influenced. He further stated that various abiotic and biotic factors such as plant age, plant parts, temperature, light, soil conditions, microflora, nutritional status and herbicide treatment influence the production and release of allelochemicals and also have influences on their phytotoxic levels or concentration. Weber and Miller, (1989) earlier stated that though chemicals in the soil solution can be absorbed by plants, they are also subject to degradation processes such as photolysis, oxidation, microbial degradation, removal and transfer processes such as volatilization, absorption etc. According to Blum (1999), once in the soil, the bioactive concentration of allelochemicals is determined by the sorption, fixation, leaching, chemical and microbial degradation. Inderjit (2001) also asserted that abiotic (physical and chemical) and biotic (microbial) factors can influence the phytotoxicity of chemicals in terms of quality and quantity required to cause injury. He stated further that these factors often limit the accumulation of allelochemicals to phytotoxic level. Stark and Hyvarinen (2003) opined that an important reason for such a reversal of response could be the role played by the soil microorganisms in detoxifying or utilizing the allelochemicals resulting in minimized plant to plant interference. The result in this study is consistent with that of Tian (1992) who stated that phytotoxic effect observed in the laboratory was not harmful to maize and cowpea growth under field condition because the phytotoxic compounds get rapidly degraded in the field than in the laboratory which resulted in a significant increase in maize and cowpea yield. Adetayo *et al.,* (2005) reported a significant increase in the vegetative growth of maize (Zea mays), cowpea (*Vigna unguiculata*), and tridax (*Tridax procumbens*) treated with aqueous extracts of *C. odorata.* Similarly, Ilori *et al.,* (2011) showed that FSEC significantly enhanced the shoot fresh and dry weight, shoot height and leaf area of *Celosia argentea.* A similar growth promoting effect of FSET on older cowpea and maize plants was reported by Oyerinde *et al.,* (2009) and Aladejimokun *et al.,* (2014).

Accumulation of chlorophyll b and total chlorophyll in the young shoot of *H. sabdariffa* subjected to FSEC and FSET treatment was significantly higher than that of the plants in the control group at $p \leq 0.05$. Earlier workers have reported similar finding (El-Darier and Youseff 2000; Dieguez-Rojo and Gonzalez 2003; Azra 2011; Ignat *et al.*, 2011; Abdalla 2014). This result also corroborates that of Otusanya *et al.* (2008, 2014) in which chlorophyll b and total chlorophyll accumulation in *Lycopersicon esculentum* and *Amaranthus dubius* plants treated with water soluble root exudate (WRE) of *T. diversifolia* was significantly enhanced at $p \leq 0.05$.

Relatively high endogenous ascorbic acid content (9.131 mg/100g) was observed for the control *H. sabdariffa* plant. The application of the two weeds aqueous extracts (100%FSEC and 100%FSET) further enhanced significantly the accumulation of this vitamin in this crop plant. High ascorbic acid concentration in the leaves and shoot of *H. sabdariffa* has been reported by earlier researchers (Morton, 1987; Amin *et al.*, 2008; Sarkiyayi and Ikioda, 2010).

In this investigation, protein percent of *H. sabdariffa* leaves was increased significantly by 100 % FSEC and 100%FSET application at $p \leq 0.05$. Ikewuchi *et al.*, (2013) reported that p-hydroxybenzoic and p-coumaric acids are two novelty phytochemicals in *C. odorata* leaves extract. Baziramakenga *et al.*, (1997) and Inderjit and Nayyar, 2002 found that p-hydroxybenzoic and p-coumaric stimulated protein synthesis by increasing the incorporation of amino acid (35 S-methionine) into protein in seedlings. Consequently, the stimulatory effect of FSEC on protein contents of *H. sabdariffa* may be due to these allelochemicals contained in the extracts. This result corroborates the findings of Al-Watban and Salama (2012) who reported that aqueous extracts of *Artemisia monosperma* aerial parts at concentrations 2.0 and 4.0% w/v increased the content of proteins in tissues of common bean seedlings. El-Khawas and Shehata, (2005) reported that leaf leachates of *Eucalyptus rostrata* and *Acacia nilotica* differentially affected the accumulation of soluble sugars and proteins in tissues of corn and common bean seedlings. In addition, Saleh (2013) demonstrated that treatment of corn seeds with olive processing waste (OPW) aqueous extract at concentration of 3.0% w/v significantly increased the soluble sugars and proteins content in seedling tissues.

CONCLUSIONS

The present study had shown that aqueous extract of *T. diversifolia* and *C. odorata* could play differing physiological role on the growth of *H. sabdariffa* plant, by inhibiting germination and juvenile seedling growth dose-dependently on one hand and stimulating the growth of older soil-cultured plants on the other hand. The result also indicated that the aqueous extracts of *T. diversifolia* was more phytotoxic than that of *C. odorata* and the growth of the radicle was more retarded by these extracts than the plumule. The stimulation of the growth of the test crop observed in soil culture experiment showed that the response of this crop to allelochemicals was a function of the age of the plant and medium of growth. The latter experiment suggested the growth promoting potential of the extracts of *C. odorata* and the advantage of using this weed either as green manure or bio-fertilizer for the cultivation of *H. sabdariffa*. Further study is recommended to identify the exact allelochemicals in the two weeds which promote the growth of the test crop.

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APPENDICES

Table 1: Effect of Different Concentrations of Aqueous Extract of *Tithonia diversifolia* (FSET) and Chromolena Odorata (FSEC) on Germination, Plumule and Radicle Length of *Hibiscus sabdariffa*

Treatments	Germination (%)	LSD P≤0.05	Plumule Length (cm)	LSD P≤0.05	Radicle Length (cm)	LSD P≤0.05
Control	97.5		3.03		2.10	
50% FSET	74.0a	0.010	1.03a	0.000	0.71a	0.010
80% FSET	67.5ab	0.000	0.78a	0.000	0.50a	0.000
100% FSET	63.0 ab*	0.000	0.57a	0.000	0.34a	0.000
50% FSEC	84.8ac	0.038	2.26b	0.050	1.96b	0.605
80% FSEC	80.0ac	0.006	2.11b	0.025	1.78b	0.246
100% FSEC	75.5a*	0.010	1.92b	0.007	1.46b	0.022

LSD ($p \leq 0.05$) = values less than or equal to 0.05 is significantly different from the control. Means followed by the same letters are not significantly different from each other at $p \leq 0.05$. * mean significantly different at 0.05 level of probability; Each value represents the mean of 6 replicates, each with 10 seeds

Table 2: Effect of Aqueous Extract of T. Diversifolia (FSET) and C. Odorata (FSEC) on the Shoot Height, Stem Girth and Number of Leaves on H. Sabdariffa

	(a) Shoot Height (cm)			(b) Stem Girth(cm)			(c) Number of Leaves		
Weeks of FSE Application	**Control**	**Fset**	**Fsec**	**Control**	**Fset**	**Fsec**	**Control**	**Fset**	**Fsec**
1	17.98	23.82	29.46	0.5	0.82	0.86	5.60	8	10
2	22.72	37.02	40.5	0.56	1.19	1.72	7.40	11.6	17.4
3	28.12	48.16	50.72	0.86	1.92	2.18	9.40	17.6	24.4
4	34.26	60.76	70.42	1.22	2.46	2.78	10.40	24.8	33.2
5	41.76	67.74	86.44	1.48	2.78	3.34	11.20	31	39.6
6	45.1	73.94	99.02	1.62	2.96	3.72	11.60	34.4	43.2
Grand Mean	31.657	51.907	62.76	1.04	2.022	2.433	9.267	21.233	27.976
LSD p<0.05		0.102	0.017*		0.061	0.012*		0.050*	0.005*

*mean significantly different from control at 0.05 level of probability; each value represents the mean of 5 replicates

Table 3: Variation in the Leaf Area, Leaf Area Ratio and Shoot Fresh Weight of H. Sabdariffa as Affected by Application of Aqueous Extract of T. Diversifolia (FSET) and C. Odorata (FSEC)

	(a) LEAF Area (cm^2)			(b) Leaf Area Ratio			(c)Shoot Fresh Weight(g)		
Weeks Of Fse Application	**Control**	**Fset**	**Fsec**	**Control**	**Fset**	**Fsec**	**Control**	**Fset**	**Fsec**
1	20.154	32.524	35.677	83.28	57.87	37.09	1.805	4.849	7.985
2	31.498	50.641	58.59	79.14	58.68	34.46	2.963	7.695	15.952
3	53.445	80.336	85.896	89.67	57.51	36.58	4.788	12.713	21.889
4	61.186	101.776	124.65	66.15	47.21	40.54	6.653	18.95	26.72
5	65.385	119.463	152.306	39.13	44.43	42.63	9.512	24.061	30.272
6	72.622	132.282	176.706	30.76	40.37	42.67	15.193	26.604	35.635
Grand Mean	50.715	86.170	105.637	64.688	51.012	38.995	6.819	15.812	22.909
LSD p<0.05		0.152	0.034*		0.135	0.009*		0.082	0.004*

*mean significantly different from control at 0.05 level of probability; each value represents the mean of 5 replicates

Table 4: Time-Course Changes in Shoot Biomass, Chlorophyll A and Chlorophyll B Accumulation of *H. sabdariffa* as Induced by Application of Aqueous Extract of *T. diversifolia* (FSET) and *C. odorata* (FSEC)

	(a) Shoot Dry Weight(g)			(b) Chlorophyll a (µM)			(c) Chlorophyll b (µM)		
Weeks of FSE Application	**Control**	**Fset**	**Fsec**	**Control**	**Fset**	**Fsec**	**Control**	**Fset**	**Fsec**
1	0.201	0.505	0.902	2.197	2.527	3.22	0.877	1.958	2.418
Table 4: Contd.,									
2	0.324	0.789	1.579	3.709	3.477	5.456	0.642	7.643	3.186
3	0.481	1.251	2.193	3.909	4.109	9.382	1.056	9.358	3.529
4	0.762	1.89	2.668	4.644	6.654	8.89	0.436	1.757	4.35
5	1.463	2.399	2.998	4.666	7.876	5.421	1.321	1.147	4.491
6	1.969	2.69	3.611	5.132	9.676	4.165	1.315	0.942	4.52
Grand Mean	0.867	1.587	2.325	4.043	5.720	6.086	0.941	3.801	3.749
LSD p<0.05		0.152	0.034*		0.216	0.136		0.043*	0.040*

*mean significantly different from control at 0.05 level of probability; each value represents the mean of 5 replicates

Table 5: Effect of Fresh Shoot Aqueous Extracts of *T. diversifolia* (FSET) and *C. odorata* (FSEC) on the Ascorbic Acid and Crude Protein Accumulation in The Young Shoot of *H. sabdariffa*

(a) Total Chlorophyll (µM) (b)Ascorbic acid (mg/100g) (c) Crude Protein (%)									
Weeks of FSE Application	**Control**	**Fset**	**Fsec**	**Control**	**Fset**	**Fsec**	**Control**	**Fset**	**Fsec**
1	3.074	4.485	5.638	6.189	7.099	7.645	4.991	6.829	7.268
2	4.351	11.12	8.642	7.281	12.195	13.651	5.166	7.968	7.53
3	4.965	13.467	12.911	9.465	14.561	17.656	6.304	8.756	8.494
4	5.08	8.411	13.24	9.465	16.746	19.112	6.742	10.683	12.88
5	5.987	9.023	9.912	10.193	17.292	21.66	7.443	12.88	12.88
6	6.447	10.618	8.685	12.195	23.116	23.116	7.968	14.097	15.411
Grand Mean	4.984	9.521	9.839	9.131	15.168	17.140	6.436	10.202	10.744
LSD p<0.05		0.007*	0.004*		0.041*	0.010*		0.027*	0.013*

*mean significantly different from control at 0.05 level of probability; each value represents the mean of 5 replicates